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EXPERIMENTS UPON THE EXTIRPATION OF THE PITUITARY AND THYROID GLANDS IN TADPOLES.

BENNET M. ALLEN.

IN recent years great interest has been aroused in the glands of internal secretion. It has been found that these play a rôle of fundamental importance in metabolism and development. It is the latter phase of the subject that has especially appealed to the author and the students whom he has started in this line of investigation. While there has been a large amount of work done upon these glands in adults, there has been comparatively little study of them in embryos. The reason is not far to seek, since our interest is chiefly stimulated by the importance of these glands to medicine. The investigators have naturally turned to the mammals for experimental study. In the study of embryology the development of the chick has chiefly attracted attention.

Now neither the mammals nor the birds nor reptiles afford ready facilities for the study of the functions of these glands during embryonic development. A few years ago Gudernatsch carried on some stimulating and suggestive work upon the effects of feeding thyroid preparations to tadpoles. He was able by this means to greatly accelerate metamorphosis, causing a precocious development into the adult condition. It occurred to the author that since such precocious development was produced by an excess of thyroid material, an inhibition of metamorphosis might be produced by the removal of the thyroid gland. This is an extremely simple process, being accomplished by making a transverse cut just back of the thyroid gland, at the beginning of its development, in *Rana pipiens* tadpoles of 6.5 mm. total length. The tadpoles thus deprived of the thyroid gland developed in a perfectly normal fashion up to the time when the hind limbs began to grow. These limbs reached a length of four to five mm. and then absolutely ceased developing. This cessation of differentiation was not only seen in the case of the limbs, but was evident in the length of the intestine, characteristics of the mouth, the persistence of the tail, and the development of the skeletal system.

Fourteen thyroidless tadpoles were found to have retained their larval condition last September, while all of the control tadpoles had metamorphosed by the 13th of August. The thyroidless tadpoles continued to grow in size, but remained absolutely stationary in their differentiation. A study was made of the thyroidless tadpoles killed from time to time, and most interesting facts were gained.

One of my students, Mr. George Terry, made a study of the skeletal system, and found that the vertebræ and the bones of the limbs remained in that stage of development that they had attained when the frog ceased to show further signs of metamorphosis.

Another student, Mr. James Rogers, studied the effects of thyroid removal upon the thymus and pituitary glands. He found that the thymus gland in the thyroidless tadpoles continued to develop after it had ceased to grow in normal controls. The pituitary gland reached at least full size in the absence of the thyroid, and apparently grew even larger than normally. These glands are all closely interrelated, and the results thus obtained are of considerable interest.

The most interesting feature was the development of the gonads and germ cells. While the body as a whole remained absolutely stationary in its stage of development, the germ glands and germ cells developed almost normally. Ripe sperm were found in tadpoles killed December 15 and February 7. The ova continued their development until they were easily visible with the naked eye. A comparison with the germ glands of young frogs that had metamorphosed last summer, and had undergone normal growth, showed no higher degree of development than that found in these thyroidless tadpoles. It is true that the latter had gonads a trifle smaller than those of the normal frogs, but this is readily explainable upon the basis of the smaller size of the body. The gonads are proportionately as large in these thyroidless tadpoles as in the control frogs of the same age. It is most significant that of all organs studied, the germ glands alone have continued in their development.

This is very beautifully substantiated in a piece of research carried out during the previous year by Mr. W. W. Swingle, who under my direction studied the effects of thyroid feeding upon the germ glands of tadpoles of this same species. He

found that by feeding in the same way that Gudernatsch had done he produced the marked acceleration of metamorphosis mentioned above, but that the germ glands and germ cells remained unaffected, continuing their normal slow growth and differentiation. Here again we have an influence exerted on every organ except the germ glands.

I think we are thus justified in saying that the germ glands are not directly influenced by the thyroid gland. They neither show acceleration with the administration of thyroid nor retardation of development in absence of the thyroid.

Investigations were made upon the effect of removal of the pituitary gland of *Rana pipiens*. This was accomplished quite readily by making an incision across the front of the head and extending just beneath the pituitary gland. The latter was then easily picked away with a needle. As a result of this the tadpoles within eight days after the operation assumed a bright silvery color throughout, due to the migration of the black pigment cells from the epidermis, and to their marked contraction throughout the whole body. This was true whether these pigment cells were located in the skin or in the internal organs. Metamorphosis was prevented just as truly as by the removal of the thyroid gland. Owing to a very high mortality brought about by the absence of the pituitary secretion, it was impossible to study the later development of these tadpoles as might be wished. Enough is known, however, to enable us to say that metamorphosis is distinctly inhibited. This might possibly be explained by the failure of the thyroid gland to undergo its full development. Experiments are being conducted now to determine this question. There is no doubt that the absence of the pituitary gland caused the tadpoles to be very susceptible to unfavorable conditions. This could not be explained by injury produced, because many tadpoles that have been operated upon failed to show the equal change and continued to develop normally, reaching a size very much greater than that of the silvery tadpoles. When these normal-appearing tadpoles were sectioned it was found that the pituitary gland had not been removed in the operation, although the injury had been just as severe in these cases as in those in which the operation had been successfully carried out. There was neither a serious retardation in growth nor an appreciably large mortality. While both of these features were mainly

true in the tadpoles where the operation had been successfully performed.

These experiments will certainly lead to a very extensive series of other investigations of similar kind. A large and extremely fruitful field of research is opened up by this work.

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THE RELATION BETWEEN THE GERM CELLS AND THE ENVIRONMENT.

BENNET M. ALLEN.

THE constitution of the germ plasm is one of the most prominent questions before biologists. This problem has been attacked by the study of cell lineage, deformation, mutilation and chemical treatment of eggs before and after fertilization; by a study of the relation between egg and sperm in fertilization, and through it all have run the brilliant developments in the field of cytology and experimental breeding. We have thus come to understand some of the cardinal facts regarding the mechanism of heredity.

The individuality of the chromosomes; their occurrence in pairs; the sex chromosomes; sex-linked characters; the fatal results of multipolar division; the explanation of mutations upon the basis of chromosomal defects and mixtures—all of these results have been interpreted to show that the potentialities of the germ plasm are laid down in the *physical* structure of the chromosomes. But through all this work we have difficulty in estimating fact and theory at their proper value. This has led to our elevating theories to the rank of established truths. Many have come to believe that the germ plasm is aloof from the soma and absolutely proof against any changes that may take place in the environment.

Without allowing ourselves to dogmatize upon this subject, let us inquire into the nature of the relation between the soma and the germ cells.

In studying the early history of the germ cells of the vertebrates, I have been struck by the fact that they undergo an orderly migration from analgen, that are often remote, toward the regions where the gonads will ultimately develop. They appear to be guided and stimulated by influences exerted by the body cells. This striking migration of the germ cells re-